

Claims

We claim:

1. A polynucleotide hybridization buffer for automated in situ hybridization comprising a low molecular weight dextran sulfate.

2. The polynucleotide hybridization buffer of claim 1 wherein said dextran sulfate has a average molecular weight of about 13,000.

3. The polynucleotide hybridization buffer of claim 1 wherein said low molecular weight dextran sulfate ranges from about 8,000 to about 16,000 MW.

4. The polynucleotide hybridization buffer of claim 1 wherein said concentration of low molecular weight dextran sulfate ranges from about 5 % to about 25 %, wt./vol.

5. The polynucleotide hybridization buffer of claim 1 wherein said buffer optionally contains formamide having a concentration of from about 5% to about 80%, wt./vol.

6. A polynucleotide hybridization buffer comprising:

a) from about 5% to about 25 % wt/vol dextran sulfate, MW 13,000;

b) from about 20% to about 50% vol/vol formamide;

c) from about 5 mM to about 10 mM Tris (15:85 of Tris-HCl:Tris-OH);

d) from about 1mM to about 5mM EDTA;

e) up to about 300 mM NaCl;

f) about 30mM trisodium citrate;

g) from about 0.025% to about 0.05% Brij-35,

adjusted to about pH 7.3.

7. A polynucleotide hybridization buffer comprising:

a) from about 10% to about 20% wt/vol dextran sulfate, MW 13,000;

b) from about 40% to about 80% vol/vol formamide;

c) about 2x SSPE; and

d) about 0.05% Brij-35.

8. A polynucleotide hybridization buffer comprising:

a) from about 10% to about 20% wt/vol dextran sulfate, MW 13,000;

5 b) about 6x SSPE; and

c) about 10% formamide.

9. A method of automatically hybridizing a polynucleotide probe to a target, comprising the steps of

10 a) preparing a section of tissue or cells to be examined;

b) hybridizing the tissue section or cellular preparation with a polynucleotide probe composition in the presence of low molecular weight dextran sulfate wherein said probe composition contains at least one sequence complementary to a coding region of the target;

15 c) removing unhybridized probe from said tissue section or cellular preparation; and

d) detecting the hybridized probe-target combination.

10. The method of claim 9 wherein said polynucleotide probe composition is selected
20 from the group consisting of DNA probes and RNA probes.

11. The method of claim 9 wherein said tissue section is a paraffin-embedded tissue section.

25 12. The method of claim 9 wherein said tissue section is a fresh-frozen tissue section.

13. The method of claim 9 wherein said polynucleotide probe composition is labeled with a detectable label.

30 14. The method of claim 9 wherein said label is selected from the group consisting essentially of fluorophores, haptens and chromogens.

15. The method of claim 9 wherein the step of preparing a section of tissue or cells to be examined comprises a liquid-based preparation step.
- 5 16. The method of claim 9 wherein the step of preparing a section of tissue or cells to be examined comprises contacting the target RNA or DNA with blocking DNA to suppress background cross-reactive signal.
- 10 17. The method of claim 9 wherein said hybridization, removal and detection steps are performed by an automated tissue staining instrument.
18. The method of claim 9 wherein said probe composition is arrayed on a solid substrate.